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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Cohen et al.  
Serial No.: 10/840008  
Filed: May 5, 2004 Docket No.: 9124.140US01  
Title: INJECTABLE CROSS-LINKED POLYMERIC PREPARATIONS AND  
USES THEREOF

CERTIFICATE UNDER 37 CFR 1.8:

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By:   
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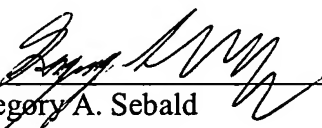
Dear Sir:

Applicants enclose herewith one certified copy of a Israel application, Serial No. 155774, filed May 5, 2003, the right of priority of which is claimed under 35 U.S.C. § 119.

Respectfully submitted,

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Patent Law, 5727 - 1967

חוק הפטנטים, תשכ"ז-1967

בקשה לפטנט

מספר: Number	155774
תאריך: Date	05-05-2003
הוקדם/נדחה Ante/Post-Dated	

Application for Patent

אני, (שם המבקש, מענו ולגבי גוף מאוגד - מקום התאגדותו)

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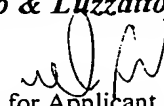
תכשירים הניתנים להזרקה של פולימרים מצולבים ושימושיהם

(בעברית)  
(Hebrew)

INJECTABLE CROSS-LINKED POLYMER PREPARATIONS AND USES THEREOF

(באנגלית)  
(English)

מבקש בזאת כי ינתן לי עליה פטנט. hereby apply for a patent to be granted to me in respect thereof.

*בקשת חלוקה - Application of Division		*בקשת פטנט מוסף - Application for Patent Addition		*דרישה דין קדימה Priority Claim	
*מבקשת פטנט from Application מס' _____ dated _____ מיום	*לבקשה/לפטנט to Patent/Appl. מס' _____ dated _____ מיום	מספר/סימן Number/Mark	תאריך Date	מדינת האיגוד Convention Country	
*יפוי כח: כללי / מיוחד - רצוף / בזה / עוד יוגש P.O.A.: general / individual - attached / to be filed later הוגש בענין 126263 filed in case					
המען למסירת מסמכים בישראל Address for Service in Israel לוצאטו את לוצאטו ת.ד. 5352 באר שבע 84152 מספרנו: 15977/03					
חתימת המבקש Signature of Applicant  Luzzatto & Luzzatto By:  Attorneys for Applicant		היום 5 בחודש מאי שנה 2003 of the year of This			
		לשימוש הלישכה			

טופס זה כשהוא מוטבע בחותם לישכת הפטנטים ומושלם במספר ובתאריך ההגשה, הינו אישור להגשת הבקשה שפרטיה רשומים לעיל.  
This form, impressed with the Seal of the Patent Office and indicating the number and date of filing, certifies the filing of the application the particulars of which are set out above.

\* מחק את המיותר Delete whatever is inapplicable

**תכשירים הניתנים להזרקה של פולימרים מצולבים ושימושיהם**

**INJECTABLE CROSS-LINKED POLYMER PREPARATIONS  
AND USES THEREOF**

### **Field of the Invention**

The present invention relates to injectable pharmaceutical preparations containing cross-linked polymer, particularly alginate, as an active ingredient, which preparations form a hydrogel *in vivo*. The invention also relates to the various uses of the injectable cross-linked alginate preparations and to methods of treatment employing the same, particularly repair of cardiac tissue damage and ablation of cardiac arrhythmias.

### **Background of the Invention**

The promising results of cardiac cell transplantation or tissue engineering in animal models have been partially attributed to reconstruction of the extracellular matrix (ECM), which maintains the structure, thickness, and elasticity of the LV wall. Manipulation of ECM by injecting alginate-based biomaterial could possibly efficiently preserve the structure and function of the left ventricular (LV) while providing a scaffold for healing and self-repair.

US Patent No. 5,709,854 describes injectable solutions of cells and polymers, which gel *in vivo*. These solutions are used for promoting tissue formation.

Injectable polymeric pharmaceutical compositions have been described. For example, US Patent No. 6,129,761 describes slowing polymerizing hydrogels which used for the delivery of cells by injection. Specifically, the publication describes cell-polymer suspensions, wherein the polymer may be, *inter alia*, an alginate, and is intended for improving the implantation of the cells.

US Patent No. 6,171,610 describes the generation of new tissue by liquid hydrogel compositions which comprise a hydrogel and tissue precursor cells.

The techniques described in these references necessarily use cells. This may be a considerable disadvantage.

An injectable biopolymer that prevents negative LV remodeling and preserves cardiac function after myocardial infarction is described by Karen L. Christman [Program of the International Conference on Engineering Tissue Growth, Pittsburg, Pennsylvania, March 17-20, 2003]. The author states that injectable fibrin glue may serve as an internal wall support and/or tissue engineering scaffold to prevent deleterious ventricular remodeling and deterioration of cardiac function. While this publication proposes to use cell free compositions, it is to be noted that *in vivo*, fibrin glue can be retained as such for only about 7 days, and, moreover, it is immunogenic. These are evident disadvantages in the rather long process of tissue regeneration.

WO97/44070 (by the present inventors) describes implantable polysaccharide, e.g. alginate, sponges for use as a matrix, substrate or scaffold for replacement or repair of tissue that has been removed or damaged. The sponges described in this publication are not injectable, and require surgical intervention. Avoiding surgery would be a great advantage.

US 5,776,445 describes an ophthalmic delivery system comprising an alginate which has a particular proportion of guluronic acid, which undergoes a change from dissolved phase to a gel phase upon contacting the lacrimal fluid.

In search for a pharmaceutical composition for promoting repair of damaged tissues, the inventors found that injectable polymeric solutions may be useful.

It is therefore an object of the present invention to provide such injectable pharmaceutical preparations, which contain non-immunogenic, non-enzymatically degradable, bio-erodible polymers.

It is a further object of the present invention to provide such injectable compositions in which the said polymer is alginate, particularly cross-linked alginate.

It is a further object the present invention to use cross-linked alginate in the preparation of injectable solutions for promoting tissue repair and regeneration.

These and other objects of the invention will become apparent as the description proceeds.

### **Summary of the Invention**

The invention relates to use of a cross-linked biocompatible polymer in the manufacture of injectable preparations for promoting repair and regeneration of damaged tissue. Preferably, the polymer is an alginate and cross-linked with calcium ions.

The use of the invention is particularly directed to the preparation of injectable preparation for promoting repair and regeneration of cardiac tissue, particularly for thickening the left ventricular wall following myocardial infarct.

The preparations prepared by the use of the invention may further optionally contain additional therapeutic agents.

In a second aspect, the invention relates to an injectable preparation comprising a cross-linked biocompatible polymer, for promoting repair and regeneration of damaged tissue. Preferably, the polymer is alginate, preferably is cross-linked with calcium ions.

The preparation of the invention are particularly intended for promoting repair and regeneration of cardiac tissue, particularly for thickening the left ventricular wall following myocardial infarct. The preparations of the invention may also be suitable for ablation of arrhythmias.

#### **Detailed Description of the Invention**

The promising results of cardiac cell transplantation or tissue engineering in animal models have been partially attributed to reconstruction of the extracellular matrix (ECM), which maintains the structure, thickness, and elasticity of the LV wall. The inventors have investigated whether manipulation of ECM by injections of alginate-based biomaterial can efficiently preserve the structure and function of the LV while providing a scaffold for healing and self-repair.

Surprisingly, the inventors found, as will be shown in the following Examples, that attenuation of LV dilatation and myocardial dysfunction following myocardial infarct by injection of a solution of cross-linked alginate, injected to infarcted myocardium in a rat model, was comparable to those achieved by embryonic cardiomyocytes transplantation.

Thus, the use of injectable polymeric solutions to treat cardiac infarcts may be an efficient replacement for the use of the difficult to obtain



embryonic cells, in the treatment of myocardial infarct (MI) and chronic heart failure (CHF).

Thus, the invention relates to use of a cross-linked biocompatible polymer in the manufacture of an injectable preparation for promoting repair and regeneration of damaged tissue.

The polymer to be used by the invention should be a biocompatible polymer, which is capable of gelling *in vivo* and thus form a hydrogel depot at the site of injections. The polymer should be non-enzymatically degradable, and bio-erodible. Furthermore, the polymer is preferably non-immunogenic. A preferred polymer to be used by the invention is alginate or modified alginate carrying sulphate groups or cell adhesion sequences. Other polymers may be, for example, polysaccharides selected from the group comprising the polyanionic polysaccharides: hyaluronic acids, gellan, gellan gum, xanthan, agar and carrageenan and the polycationic polysaccharide: chitosan; as well as synthetic organic polymers such poly(phosphazenes), polyethylene oxide and its copolymers with polypropylene glycol, poly(acrylic acids), poly(methacrylic acids), poly(vinyl acetate) and sulfonated polymers.

Cross-linking may be achieved by using ions, altering the pH or changing the temperature. Ionic cross-linkers include metal cations, such as calcium, copper, aluminum, magnesium, strontium, barium, tin, zinc, chromium, di-, tri- and tetrafunctional organic cations; or anions selected from the group consisting of low molecular weight dicarboxylic acid, sulfate ions and carbonate ions. Polyions may be used such as poly(amino acids), poly(ethyleneimine), poly(vinylamine), poly(allylamine), and polysaccharides.

It is to be noted that the degree of cross-linking would determine the rate of erosion of the solid depot formed *in vivo* upon injection. The concentration of the polymer in the injectable solution would depend on its molecular weight and on the intended degree of cross-linking. The design of the preparation should take into account the factors necessary to achieve an adequate liquid/solid phase transition. Such design is within the capabilities of a person skilled in the art of pharmacy.

The injectable preparations prepared by the method of the invention are particularly suitable for the treatment of damaged cardiac tissue, following myocardial infarct, and/or for the treatment of chronic heart diseases. In particular, the injectable preparations manufactured by the method of the invention are intended for thickening the left ventricular wall following a myocardial event. Additionally, it is expected that the injectable preparations of the invention may be suitable for the treatment of tissue damage resulting from arrhythmia. Using electrochemical mapping, the injectable preparation of the invention can be used for "bio-ablation", i.e. ablation of cardiac arrhythmias by injection into arrhythmic foci and pathways.

The injectable compositions of the present invention may optionally contain additional therapeutic agents. Such agents may be, for example, various growth factors such as, angiogenesis stimulating factors and revascularization enhancing factors (e.g. basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), members of the TGF-family, bone morphogenic proteins (BMP), platelet-derived growth factors, and other agents such as myogenic factors, transcription factors, cytokines, and homeobox genes.

The invention will be described in more detail on hand of the following Examples, which are illustrative only and do not limit the invention, which is only defined by the appended claims.

## **Examples**

### ***Methods and Results:***

#### **Preparation of the injectable alginate biomaterial:**

Sodium alginate (MW ranging between 3 – 100 kDa) is dissolved in double distilled water, to a final concentration of 2% (w/v) and filtered through 1.2, 0.45 and 0.2 $\mu$ m nylon membrane filters. The alginate solution is cross-linked with calcium ions by adding 2% (w/v) calcium gluconate solution, while stirring intensively. The viscosity of the cross-linked alginate solution can be manipulated by changing the weight ratio of calcium ions and alginate, as well as by judiciously selecting polymer molecular weight and composition (M/G ratio), nonetheless, in all cases the cross-linked alginates solution is of a viscosity that allows it to be injected.

#### **In vivo studies**

Seven days after extensive MI, rats were randomized to alginate-based biomaterial injections, embryonic cardiomyocyte ( $1.5 \times 10^6$ ) implantation, or medium injection into the myocardial scar. The alginate biomaterial was calcium cross-linked, yet it still flows under injection conditions, as described above. Echocardiography study was performed before and 1 and 2 months after implantation to assess left ventricular (LV) remodeling and function. Hearts were harvested 2 months after implantation for histological evaluation.

Serial echocardiography studies revealed that the alginate-based biomaterial injection enhanced scar thickness, prevented LV dilatation and dysfunction, comparable to cardiac cell transplantation, while control

animals developed significant LV dilatation accompanied by progressive deterioration in LV contractility.

The results are summarized in Table 1.

**Table 1**

	Alginate (n=7)		Cells (n=5)		Medium (n=4)	
	Before	2m After	Before	2 m After	Before	2 m After
2-D Echo						
LVDD mm	0.70±0.02	0.85±0.05	0.68±0.03	0.69±0.01	0.73±0.04	0.97±0.04*
LVSD mm	0.52±0.05	0.65±0.06	0.45±0.02	0.45±0.04	0.56±0.04	0.80±0.04*
LVSA mm	0.22±0.03	0.34±0.06	0.17±0.02	0.18±0.02	0.23±0.3	0.42±0.03*
LV FS%	27±5	25±4	33±3	34±6	23±3	17±1*

\*2 months after vs. before; p<0.05

LVDD-LV diastolic dimension, LVSD- LV systolic dimension, LVSA-LV systolic area; FS- Fractional shortening.

**Conclusions:** The data suggest that injections of alginate-based biomaterial into the infarcted myocardium in a rat model attenuate LV dilatation and myocardial dysfunction. These results are comparable to those achieved by embryonic cardiomyocytes transplantation. The results suggest a viable alternative to the difficulties in achieving appropriate cells to treat MI and CHF.

**Claims:**

1. Use of a cross-linked biocompatible polymer in the manufacture of an injectable preparation for promoting repair and regeneration of damaged tissue.
2. The use of claim 1, wherein said polymer is alginate.
3. The use of claim 2, wherein said alginate is cross-linked with calcium ions.
4. The use of any one of claims 1 to 3, wherein said tissue is cardiac tissue, particularly the left ventricular wall.
5. The use of claim 4, wherein said damage is myocardial infarct.
6. The use of any one of claims 1 to 5, wherein said preparation further optionally contains additional therapeutic agents.
7. An injectable preparation comprising a cross-linked biocompatible polymer, for promoting repair and regeneration of damaged tissue.
8. The preparation of claim 7, wherein said polymer is alginate.
9. The preparation of claim 8, wherein said alginate is cross-linked with calcium ions.
10. The preparation of any one of claims 7 to 9, wherein said tissue is cardiac tissue, particularly the left ventricular wall.
11. The preparation of claim 11, wherein said damage is myocardial infarct.

12. The preparation of any one of claims 7 to 11, further optionally contains additional therapeutic agents.

לוצאטו את לוצאטו  
LUZZATTO & LUZZATTO  
By 